

## Quality Assurance Project Plan (QAPP) for shipboard tests of ballast water management systems

### Introduction

In this QAPP, vendor means OceanSaver AS. Further, the sampling crew consisted of; Helge Botnen (UNIFOB); Kristin Østby-Berg (UNIFOB) and Stephan Gollash (GoConsult, engaged by UNIFOB). Stephan Gollash was appointed as sampling crew leader.

### Purpose

The purpose of this document is to provide a description of the onboard testing of a ballast water treatment system according to Annex 3 Resolution MEPC.125(53) Guidelines for approval of ballast water management systems (G8) adopted by IMO on 22 July 2005. This QAPP contains requirements concerning sampling personnel, sampling equipment requirements, sampling equipment checklist, sampling procedure and the sample processing procedure.

### Shipboard test

One test cycle includes: 1) uptake of ballast water, 2) treatment of ballast water, 3) storage of ballast water on the ship (holding time), and 4) discharge of ballast water. Tests should only be carried out while the ballast water system is operating normally. All tests should include both a control ballast water tank and a treated ballast water tank. Ideally both tanks should be filled simultaneously. The position and time of ballast water uptake for both tanks and weather conditions during uptake should be recorded. Possible reasons for the occurrence of an invalid shipboard test should be reported.

### D-2 standard:

Regulation D-2 (performance standard) stipulates that ships meeting the requirements of the Convention must discharge;

- less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension;
- less than 10 viable organisms per millilitre less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and
- less than the following concentrations of indicator microbes, as a human health standard:
  - Toxicogenic *Vibrio cholerae* (serotypes O1 and O139) with less than 1 Colony Forming Unit (CFU) per 100 millilitres or less than 1 CFU per 1 gramme (wet weight) of zooplankton samples;
  - *Escherichia coli* less than 250 CFU per 100 millilitres; and
  - Intestinal *Enterococci* less than 100 CFU per 100 millilitres.

### **Sampling programme**

The sampling should focus on the following biological parameters: organisms larger than or equal to 50 µm in minimum dimension, organisms less than 50 µm in minimum dimension and greater than or equal to 10 µm in minimum dimension and bacteria, i.e. *E. coli* and *Enterococcus*. Samples for *Vibrio cholera* should be taken and treated for further DNA analysis in a land based laboratory.

In addition, the following abiotic water parameters should be measured: salinity, temperature, particulate organic carbon and total suspended solids.

**NB:** Valid tests are indicated by the abundance of organisms in uptake water and in discharge water. The abundance in **uptake water**, for both the control tank and ballast water to be treated must contain viable organism concentration exceeding 10 times the values of Regulation D-2.1<sup>1</sup>. **On discharge** the control tank must contain viable organism concentration exceeding the values of Regulation D-2.1<sup>2</sup> and the treated water must contain less viable organisms than stated in Regulation D-2.

### ***Sampling Control Water***

During uptake: One continuous sample during the entire uptake time of the control water.<sup>3</sup>

During discharge: One continuous sample during the entire discharge time of the control water.

### ***Sampling Treated Water***

During uptake: One continuous sample should be taken over the entire ballast water uptake time.

During discharge: Three replicate continuous samples during the entire discharge time of the treated water should be taken as replicates.<sup>4</sup>

### ***Sample sizes***

For all sampling events, i.e. during uptake and discharge of control and treated water, the following sample volumes should be taken. For the enumeration of **organisms greater than or equal to 50 µm** in minimum dimension, samples of **at least one cubic metre should be collected**. If samples are concentrated for enumeration the samples should be concentrated using a sieve no greater than 20 µm mesh in diagonal dimension.

For the enumeration of **organisms greater than or equal to 10 µm and less than 50 µm** in minimum dimension, samples of **at least 10 litres should be collected**. If samples are concentrated for enumeration the samples should be concentrated using a sieve no greater than 10 µm mesh in diagonal dimension.

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<sup>1</sup> At least 100 viable organisms per cubic metre for organisms above 50 micron in minimum dimension and at least 100 viable organisms per millilitre of organisms below 50 micron and above 10 micron in minimum dimension.

<sup>2</sup> At least 10 viable organisms per cubic metre for organisms above 50 micron in minimum dimension and at least 10 viable organisms per millilitre of organisms below 50 micron and above 10 micron in minimum dimension.

<sup>3</sup> By doing so the required three replicate samples of influent water, collected over the period of uptake (e.g. 1 x beginning, 1 x middle, 1 x end) is not needed, but one sample taken over the entire uptake time.

<sup>4</sup> By doing so the required three times three replicate samples of discharge water (e.g. 3 x beginning, 3 x middle, 3 x end) is not needed, but three samples taken over the entire discharge time.

For the evaluation of **bacteria**, a sample of at least **500 millilitres** should be taken.

### ***Sample processing***

Samples for **organisms greater than or equal to 50 µm** in minimum dimension need to be concentrated for enumeration by using a sieve no greater than 20 µm mesh in diagonal dimension. When concentrating samples the volume of the concentrate should be approx. 100 ml of which at least 5 ml should be analysed by using the stereomicroscope.

The life/dead judgement should be undertaken by exposure of the organisms to light (under the stereomicroscope) and in additional non-moving organisms should be poked with a needle to initiate movement. Intact and moving organisms should be considered as living, organ activity of intact organisms gives an additional indication of viability. This assessment should be undertaken onboard no later than 6 hours after sampling. Numbers of individuals are recorded along with major taxonomic groups.

Samples for **organisms greater than or equal to 10 µm and less than 50 µm** in minimum dimension need to be concentrated for enumeration by using a sieve no greater than 10 µm mesh in diagonal dimension.

A subsample of 1 litre should be concentrated to approx 50-100 ml. One set of the samples should be kept unpreserved and stored in a fridge. A second set should be preserved with Lugol Solution. Both sets of samples should be transported to the land-based laboratory without delay following the Sample Transfer Document (Appendix 2).

Samples for *E. coli* and Enterococci should be analysed onboard. A subsample of approx. 100 ml should be taken and filtered. Thereafter, the filter should be placed on a selective medium; one set for each bacteriae group and incubated onboard.

Samples for *Vibrio cholerae* should be transported to the land-based laboratory without delay following the Sample Transfer Document (Appendix 2).

### **Personnel and responsibilities**

The work load during sampling and sample processing requires a sampling crew of at least three trained and experienced members, and it is recommended that each have their own specialities on sample processing. It is therefore recommended that the sampling crew consist of one zooplankton specialist, one phytoplankton specialist and one microbiologist. Each sampling crew member is responsible to bring the equipment needed for the onboard analysis. One sampling team member may be replaced by a generalist, particularly if some of the samples will be processed in a land based laboratory.

All sampling crew members planning to send samples to land-based laboratories should follow the Sample Transfer Document (see Appendix 2).

Within the sampling crew one member should be appointed as leader (see responsibilities below).

During each test cycle the sampling crew will make sure that the sampling equipment is cleaned prior each sampling event, that the equipment is correctly installed, that the samples are taken from correct sampling points, that the necessary number and volume of samples are correctly taken and that sample handling and sample processing is correctly performed.

*Responsibilities of the sampling crew leader*

- All communication concerning the test between sampling crew and the crew of the treatment system vendor/ship crew
- In close consultation with the crew of the treatment system vendor/ship crew the sampling crew leader will decide
  - when to start ballast water uptake
  - when to start the sampling event
  - when to finish the sampling
  - when to start the ballast water discharge
  - when to start the ballast water sampling during discharge
  - when to finish the sampling event
- Responsible for shipment of samples, if necessary
- Responsible for reporting of the results

The leader of the sampling crew can transfer responsibilities or duties to other sampling crew members.

*Responsibility of specialist for organisms' larger than 50  $\mu\text{m}$*

- Prepare all equipment for sampling
- Prepare all equipment for sample processing
- Process all samples
- Reporting of results to the sampling crew leader

*Responsibility of specialist for organisms' between 10- 50  $\mu\text{m}$*

- Prepare all equipment for sampling
- Prepare all equipment for sample processing
- Process all samples
- Reporting of results to the sampling crew leader

*Responsibility of microbiological specialist (bacteria)*

- Prepare all equipment for bacteria sampling
- Prepare all equipment for bacteria sample processing
- Process all bacteria samples
- Reporting of results to the sampling crew leader

*Responsibility of generalist*

- Support the installation of sampling equipment
- Measure temperature
- Measure salinity
- Collect sample for suspended particulate matter
- Collect sample for total organic content
- Document other relevant data, such as start and end time of the sampling event
- Optional photo documentation of the sampling event
- Reporting of results to the sampling crew leader

**Reporting of test results**

After each test cycle a test cycle report will be prepared by the sampling crew leader (see Appendix 3).

### **Sampling equipment, check lists, for one test**

**NB:** Unintentional contamination of sample equipment must be avoided. Remember proper cleaning of all sampling devices between sampling sessions. When possible, sample and process organism-poor water before organism-rich water to avoid contamination with organisms between samples.

In case "fragile" gear is planned to be used it is recommended to bring replacement material in case material breaks or becomes otherwise unusable.

#### *Bacteria sampling and processing*

Samples for *E. coli* and Enterococci are processed onboard according to the instructions following e.g. the kits from Dr. Möller & Schmelz.

At least six 500 ml bottles for collection of ballast water sample

Two incubators with adjustable temperature and thermometers

Dr. Möller & Schmelz, *Enterococci* – selektiv, kit

Dr. Möller & Schmelz, Lak.-ttc-tergitol-nps, kit

Trypton and Kovacs, for confirmation

Distilled water for dilution

Filtering device for filter (diameter 47 mm), must have capacity for 500 ml

Vacuum pump with hoses

GF/C filters for collection of possible *V. cholera*

Alcohol for disinfection of equipment

Forceps

Pipette with plastic tips, 0.1, 1 and 10 ml

Latex gloves

Freezer for deep freezing of filters with *Vibrio cholera* samples. Samples for *V. cholerae* will be transferred to a land-based laboratory for later analysis.

#### *Smaller plankton (10-50 µm)*

At least six 100 ml bottles for ballast water samples

Lugol

Formalin

Pipette for adding Lugol/Formalin to the sample

#### *Larger plankton (larger than 50 µm) sampling and processing*

Four filtering bags with at least eight removable and capable cod-ends

Each filtering bag equipped with an inline flow meter, for measurement of water volume

Replacement filtering mesh (one for each cod-end)

Buckets

Wash bottle

Small sieves with removable plankton mesh (one for each sample)

Zooplankton counting chambers

Dissecting microscope with light

Forceps

Needles for poking (to aid the life/dead judgement)

#### *TSS, TOC, temperature and salinity*

At least six plastic bottles for collection of ballast water

Filtering device for filter (diameter 47 mm)

Vacuum pump with hoses  
GF/C filters  
Wash flask  
Forceps  
Aluminium foil  
Small plastic bags  
Volume measurer  
Thermometer  
Refractometer

Temperature must be recorded while sampling, salinity might be recorded by the refractometer after the sampling event, samples for TSS are collected on GF/C filters which are frozen onboard and brought to a land based laboratory for processing. Filters are dried at 105 °C for approximately 20 hours and then burned at 550 °C for 2 hours. Cold filters are weighed after drying and after burning. TOC is measured in an accredited land based laboratory.

*Additional requirements for all samplings*

The sampling points must be correctly installed on the pipeline by the vendor  
All sampling points should be of identical design  
Hoses for water transfer from sampling point to position for sample collection  
Where appropriate, water tanks and a draining system at the position of the sample collection point  
Watch  
Paper and writing utensils  
Water proof marker  
Suitable boxes, sampling bottles, buckets for transportation of sampling equipment, including Styrofoam boxes with cooling unit

*Work cloths*

Light boiler suit  
Hearing protection  
Suitable foot ware  
Gloves  
Hard hat

***Transfer of samples to any land based laboratory***

In case of sample transfer, a transfer document must be issued. The document must state all parties being involved in the transfer, type of sample (water, filter etc.), number of samples, type of analysis (phytoplankton, TSS etc.), date of transfer and signature of all party representatives. The sample recipient laboratory should copy the signed sample transfer document and make this available to the sampling crew. The transfer document must be kept in the archives of the sampling crew.

## Appendix 1

**Stephan Gollasch**, marine biologist (PhD), born in Hamburg, Germany in 1962. Current position: senior scientist at GoConsult, an independent consultancy company based in Hamburg, Germany ([www.gollaschconsulting.de](http://www.gollaschconsulting.de)).

Since 2002 Gollasch is involved in various projects and programmes dealing with ballast water treatment systems, including land-based and ship-board efficacy tests. Other fields of expertise include risk assessment and the development of ballast water management scenarios. Since 1994 Gollasch is member of the German Delegation at IMO MEPC and represents his country in the Ballast Water Working Group.

**Helge Botnen**, Cand. Scient, marine biologist, born in Bergen, Norway in 1959  
Current position: Scientist and quality manager at Section of Applied Environmental Research, at UNIFOB AS, High Technology Centre in Bergen, Thormøhlensgate 49, 5006 Bergen, Norway (<http://www.sammarin.unifob.uib.no/> and <http://www.unifob.uib.no/>).

Botnen has been involved in several national and international biological surveys of ballast water and sediments from ballast water tanks since 1996. He has also conducted land based tests of concepts of ballast water treatment systems and ship board tests of a ballast water system.

**Kristin Østby-Berg**, Bachelor, food technology, born in Sarpsborg, Norway in 1982  
Current position: Engineer at AnalyCen AS, Møllebakken 50, 1538 Moss, Norway.

Østby-Berg has worked with microbiological sample processing in laboratories since 2006.

**Appendix 2****Sample Transfer Document****Project number:**

The responsibility of these samples is transferred to the recipient by the below signature of the recipient.

Owner of sample(s) and results: Stephan Gollasch, GoConsult, Grosse Brunnenstr. 61, 22763 Hamburg, sgollaschaol.com, Tel +49 40 390 54 60

Recipient of sample(s): Marcel Veldhuis, NIOZ, Texel, The Netherlands

***1 L concentrated via 10 µm. PLEASE MEASURE VOLUME OF EACH SAMPLE !!!***

Number of sample(s): 24

Sample labelling:

<b>Sample type</b>	<b>Sample label</b>	<b>Sample type</b>	<b>Sample label</b>
Phyto-plankton	BW uptake, control, south. Red Sea, Nov. 14.07	Phyto-plankton	BW uptake, control, south. Oman, Nov. 16.07
Phyto-plankton	BW uptake, treated, south. Red Sea, Nov. 14.07	Phyto-plankton	BW uptake, treated, south. Oman, Nov. 16.07
Phyto-plankton	BW uptake, control, south. Red Sea, Nov. 14.07 with Lugol	Phyto-plankton	BW uptake, control, south. Oman, Nov. 16.07 with Lugol
Phyto-plankton	BW uptake, treated, south. Red Sea, Nov. 14.07 with Lugol	Phyto-plankton	BW uptake, treated, south. Oman, Nov. 16.07 with Lugol
Phyto-plankton	BW discharge, control, Nov 16.07	Phyto-plankton	BW discharge, control, Nov 17.07
Phyto-plankton	BW discharge, treated 1, Nov 16.07	Phyto-plankton	BW discharge, treated 1, Nov 17.07
Phyto-plankton	BW discharge, treated 2, Nov 16.07	Phyto-plankton	BW discharge, treated 2, Nov 17.07
Phyto-plankton	BW discharge, treated 3, Nov 16.07	Phyto-plankton	BW discharge, treated 3, Nov 17.07
Phyto-plankton	BW discharge, control, Nov 16.07 in Lugol	Phyto-plankton	BW discharge, control, Nov 17.07 in Lugol
Phyto-plankton	BW discharge, treated 1, Nov 16.07 in Lugol	Phyto-plankton	BW discharge, treated 1, Nov 17.07 in Lugol
Phyto-plankton	BW discharge, treated 2, Nov 16.07 in Lugol	Phyto-plankton	BW discharge, treated 2, Nov 17.07 in Lugol
Phyto-plankton	BW discharge, treated 3, Nov 16.07 in Lugol	Phyto-plankton	BW discharge, treated 3, Nov 17.07 in Lugol

Date and signature of sample owner: Nov. 19.07

Date and signature of sample recipient:

**Appendix 3**

## Test Cycle Report

### Ballast Water Treatment System

Treatment system: OceanSaver®

Ship name: Höegh Trooper

Date and time for ballast water uptake: November 14, 2007, 17:31-18:55

Position of ship during ballast water uptake: 13°27,5'N 042°39,0'E - 13°15,2'N 042°58,0'E.

Distance travelled during uptake was 22.3 nm. Water depth 54 to 224 m. Distance to nearest main land 16-20 nm.

Date and time for ballast water discharge: November 16, 2007, 09:19-10:31

Position of ship during ballast water discharge: 16°00,4'N 053°36,4'E - 16°09,3'N

053°57,0'E. Distance travelled during discharge was 21.8 nm. Water depth 2425 to 2590 m. Distance to nearest main land 41.3-46.0 nm.

Holding time of ballast water between uptake and discharge (hours): approximately 41

Weather conditions during the test: Good

**Water quality and number of organisms in uptake water**

	Temp °C	Salinity PSU	TOC mg/l	TSS mg/l	<50µm organisms/m <sup>3</sup>	10-50µm organisms/ml	E.coli cfu	Enteroc. cfu	V.cholera cfu
Control	28,0	37	1.9	114	1549	NIOZ	0	0	0
Treated	28,0	37	1.5	115	841	NIOZ	0	0	0

**Water quality and number of organisms in discharge water**

	Temp °C	Salinity PSU	TOC mg/l	TSS mg/l	<50µm organisms/m <sup>3</sup>	10-50µm organisms/ml	E.coli cfu	Enteroc. cfu	V.cholera cfu
Control	29,5	37	1.3	115	332	NIOZ	0	0	0
Treated # 1	30,0	37	1.3	118	0	NIOZ	0	0	0
Treated # 2	30,0	37	1.4	115	0	NIOZ	0	0	0
Treated # 3	30,0	37	1.1	118	0	NIOZ	0	0	0

Remarks: The 10-50µm category organisms (phytoplankton) was analysed at the NIOZ laboratories and are reported upon separately.

Date and signature of the sampling crew leader

22. Dec. 2007



S. Gollasch

## Test Cycle Report

### Ballast Water Treatment System

Treatment system: OceanSaver®

Ship name: Höegh Trooper

Date and time for ballast water uptake: November 16, 2007, 21:11-22:11.

Position of ship during ballast water uptake: 17°42,3'N 056°53,6'E - 17°56,4'N 057°07,3'E.

Distance travelled during uptake was 19.3 nm. Water depth 73 to 85 m. Distance to nearest main land 32.5-35.0 nm.

Date and time for ballast water discharge: November 17, 2007, 19:12-20:25.

Position of ship during ballast water discharge: 23°15,7'N 059°13,3'E - 23°30,5'N

058°53,3'E. Distance travelled during discharge was 23.7 nm. Water depth 225 to 1359 m.

Distance to nearest main land 7-13 nm.

Holding time of ballast water between uptake and discharge (hours): approximately 24

Weather conditions during the test: Good

#### Water quality and number of organisms in uptake water

	Temp °C	Salinity PSU	TOC mg/l	TSS mg/l	<50µm organisms/m <sup>3</sup>	10-50µm organisms/ml	E.coli cfu	Enteroc. cfu	V.cholera cfu
Control	28,9	38	1.8	112	2208	NIOZ	0	0	0
Treated	28,9	38	1.6	116	365	NIOZ	0	0	0

#### Water quality and number of organisms in discharge water

	Temp °C	Salinity PSU	TOC mg/l	TSS mg/l	<50µm organisms/m <sup>3</sup>	10-50µm organisms/ml	E.coli cfu	Enteroc. cfu	V.cholera cfu
Control	29,2	38	1.3	112	361	NIOZ	0	0	0
Treated # 1	29,2	38	1.5	122	0	NIOZ	0	0	0
Treated # 2	29,2	38	1.4	117	0	NIOZ	0	0	0
Treated # 3	29,2	38	1.4	113	0	NIOZ	0	0	0

Remarks: The 10-50µm category organisms (phytoplankton) was analysed at the NIOZ laboratories and are reported upon separately.

Conclusion: Due to a change in the ships operational procedures, a decision was made by the engineer on duty to shift HFO (Heavy Fuel Oil) from starboard bunker fuel oil tank to port settling tank. Due to a consequential occurring heel to port, the ballasting of the port side tank (test tank), was abounded. The test cycle did however proceed as planned, however, the sampled volume of water was somewhat reduced (to approximately 700 l). The G8 guideline specifically notes that shipboard tests shall not interfere with normal shipboard ballasting routines. Thus, the test should be considered valid despite the reduced sampled volume of water.

Date and signature of the sampling crew leader

22. Dec. 2007



S. Gollasch

